Current Pharmaceutical Design, 1998, 4, 37-52

Animal Models of Atherosclerosis and Interpretation of Drug Intervention Studies

Thomas M.A. Bocan

Department of Vascular and Cardiac Diseases, Parke-Davis Pharmaceutical Research, Division of Warner Lambert Company, 2800 Plymouth Road, Ann Arbor, MI 48105, USA

Abstract: Atherosclerosis has often been defined as a multifactoral disease; however, a common risk factor associated with accelerated vascular disease in man or animals is an elevated plasma cholesterol level. Even though there is no one perfect animal model that completely replicates the stages of human atherosclerosis, cholesterol feeding and mechanical endothelial injury are two common features shared by most models of atherosclerosis. The models may differ with respect to degree of dietary cholesterol supplementation, length of hypercholesterolemia, dietary regimen and type, duration and degree of mechanical endothelial injury. With the advent of genetic engineering, transgenic mouse models have supplemented the classical dietary cholesterol induced disease models such as the cholesterol-fed hamster, rabbit, pig and monkey. The desire to limit the progression of atherosclerosis has spawned numerous drug intervention studies. Biochemical as well as morphologic and morphometric changes in the extent, structure and composition of atherosclerotic lesions following drug intervention have become major endpoints of in vivo drug intervention studies. Interpretations of alterations in vascular pathology following drug administration are often confounded by associated changes in plasma lipids and lipoproteins, limitation of the animal models and additional properties of compounds unrelated to their primary mode of action. Thus, the current review will summarize the pathology of atherosclerosis, describe various animal models of vascular disease and provide a critical review of the methods utilized and conclusions drawn when evaluating pharmacologic agents in animals.

Introduction

Atherosclerosis has often been defined as a multifactoral disease. In addition, hypercholesterolemia has become a widely accepted risk factor for premature development of coronary artery disease. Classical thinking argued that development of clinically significant atherosclerotic lesions was associated with two major processes. One is fibrocellular proliferation, which adds to intimal bulk and eventually leads to chronic ischemic syndromes via gradual constriction of the arterial lumen. The second process involves the combination of cellular necrosis and lipid deposition within the arterial intima. Enlargement of a lipid-rich core tends to erode the fibrous cap [1], eventuating in plaque rupture, exposure of circulating blood to highly thrombogenic material and sudden ischemic episodes such as myocardial infarction [2,3]. Considering our classical understanding of atherosclerosis progression, the current article will review the histologic landmarks of the various stages of atherosclerosis and also provide a dynamic understanding of how the stages might be interrelated. A comparison of various hypercholesterolemia-induced animal models of atherosclerosis will be made with a focus on their advantages and limitations when used to evaluate novel antiatherosclerotic drugs. Finally, the antiatherosclerotic activity of inhibitors of acyl-coenzyme A:cholesterol O-acyltransferase (ACAT) Fig. (1) (1-14), 3-hydxroxy-3-methylglutaryl coenzyme A (HMG-CoA reductase) Fig. (2) (15-18), 15-lipoxygenase (15-LO) and lipoprotein oxidation (anti-oxidants) Fig. (3) (19-22) will be discussed; however, an emphasis will be on describing how the models can discern the compound's *direct* from *indirect* antiatherosclerotic activity.

1381-6128/98 $15.00+.00

Pathology of Atherosclerosis

Atherosclerosis is a focal disease that has been shown to develop in a distinct pattern in both man and animals [4,5]. As depicted in Fig. (4), atherosclerotic lesion development can be divided into six histologically distinct stages or lesion types and five dynamic phases [6,7]. The formation of an intimal cushion at distinct sites within the arterial tree appears to precede the development of atherosclerosis and may be considered a normal aging process. Smooth muscle cells (SMC) migrate from the media, proliferate in the intima and secrete extracellular matrix. Extracellular lipid accumulation that is primarily of lipoprotein origin [8] decorates the secreted collagen, elastin and proteoglycans of the developing intima. Oxidation of the insudant lipoproteins [9] appears to set up a chemotactic gradient and stimulate endothelial cells to upregulate adhesion molecules, i.e., vascular cell adhesion molecule-1 (VCAM-1) [10], responsible for the recruitment of monocyte-macrophages. Monocyte-macrophages are a hallmark of Type I to III lesions and are both a culprit cell responsible for promoting lesion development and a potential point of therapeutic intervention. The major difference in Type I to III lesions lies in the relative amounts of monocyte-macrophage foam cells, SMC, extracellular matrix and lipid and the gross extent of these lesions on the arterial surface. These lesions have classically been termed fatty dots, fatty streaks or fibrolipid lesions to denote their relative extent and degree of fibrosis. Therefore, progression from the innocuous intimal cushion to the Type III lesion that may occur over the first 20 to 30 years of life can be characterized as Phase 1.

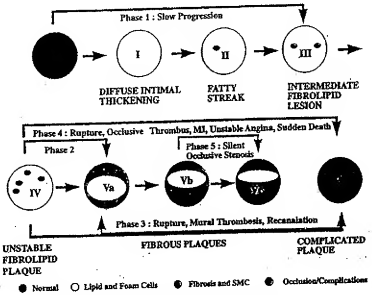


Fig. (4). Schematic representation of atherosclerotic lesion progression (adapted from references [6, 7]).

cholesterol and 4 to 8% fat [13] for 6 to 8 weeks has resulted in marked elevations in plasma cholesterol, i.e., 1000 to 3000 mg/dl, cholesteryl ester accumulation in hepatic and peripheral tissues [14,15] and development of aortic macrophage foam cell enriched lesions. Development of atherosclerosis in the coronaries is limited to the small intramyocardial vessels and not the larger epicardial vessels as has been found in man [16]. The rabbit atherosclerotic lesions were reminiscent in cellular composition to Type I to III human lesions. Kritchevsky and colleagues have performed numerous cholesterol feeding experiments in which they maintained the cholesterol supplementation constant, i.e., 2%, and altered the type of dietary fat to further refine the role of cholesterol metabolism in atherosclerosis progression. A notable finding was that upon addition of 6% peanut oil or 6% coconut oil to a 2% cholesterol diet two histologically distinct atherosclerotic lesions developed [17,18]. Peanut oil supplementation produced aortic lesions that contained relatively little lipid but abundant smooth muscle cell proliferation and collagen deposition. In contrast, addition of 6% coconut oil to the diet resulted in lesions with demonstrable intracellular lipid and intimal proliferation; however, less collagen and elastin were evident. Although elevated plasma cholesterol levels induce atherosclerotic lesions and dietary fat composition may affect the cellular composition of the lesion in rabbits, prolonged hypercholesterolemia results in exponential cholesterol enrichment of many peripheral organs [19].

The marked cholesteryl ester enrichment of peripheral organs such as the liver and spleen may be problematic when evaluating pharmacologic agents. Liver metabolism of compounds may be compromised or enhanced in animals fed a high cholesterol diet and the resulting plasma levels may be either an underestimate or overestimate of the actual efficacious drug levels. Feeding a 2% cholesterol diet results in marked plasma total cholesterol levels and cholesteryl ester enriched beta-VLDL as the primary plasma lipoprotein [20]. Given the pro-atherogenic nature of beta-VLDL [21], the direct antiatherosclerotic activity of compounds with mechanisms unrelated to cholesterol lowering may be masked due to the profound effect of beta-VLDL on monocyte-macrophage cholesteryl ester enrichment. We have noted that while compounds like ACAT inhibitors Fig. (1) (1-14) which prevent the accumulation of cholesteryl esters are antiatherosclerotic under such conditions, the 15-LO inhibitor, PD146176 Fig (3) (19), lacks activity because it's mechanism of action may be related to oxidation of lipoproteins or pre-macrophage events such as monocyte adherence and transmigration.

Rabbit models of atherosclerosis have been developed which limit the amount of dietary cholesterol supplementation [22]; however, such models are time consuming and for that reason may have limited utility for screening antiatherosclerotic agents. Wilson and colleagues [22] fed rabbits an agar-gel diet containing 19% butter and 1% corn oil for up to 5 years. Plasma total cholesterol levels were approximately 300 mg/dl and over the course of 5 years atherosclerotic lesions representing Type I to V lesions were noted. Advanced atherosclerosis can also be induced in a shorter time fraime by intermittent feeding of a 1% cholesterol, 5% cottonseed oil diet for 2 months followed by 6 months of a chow diet and 2 additional months of the cholesterol diet [23,24]. While plasma cholesterol levels fluctuated with dietary cholesterol supplementation, the five stages of atherosclerosis were present in both aorta and coronary arteries. Protracted feeding of a low cholesterol diet or intermittent feeding of high and low cholesterol diets produced histologically similar atherosclerotic lesions. Given the disparate plasma total cholesterol levels, these data suggest that the lipoprotein profile may play an important role in the rate at which atherosclerotic lesions develop. Feeding studies have indicated that beta-VLDL was the primary lipoprotein in rabbits fed a cholesterol diet while LDL-like particles predominated in animals fed a semisynthetic casein-enriched diet [25]. Morphologic and morphometric analysis of rabbits fed either a 0.125% to 0.5% cholesterol or casein-enriched diet for 6 months revealed that atherosclerotic lesions developed in both models; however, the nature and extent of lesions varied [25, 26]. At comparable plasma cholesterol levels, the cholesterol-fed

rabbits had approximately twice the extent of aortic atherosclerosis relative to the casein-fed animals and Type IV-V lesions predominated. In the casein-fed rabbits, 30% of the aorta contained atherosclerotic lesions that ranged in appearance from fatty dots to fibrous plaques with a necrotic lipid-rich core [25,26]. These data indicate that under a similar time frame and plasma cholesterol level the type of dietary supplementation can affect the quantity and type of atherosclerotic lesion that develops primarily by altering the major cholesterol caring lipoprotein, i.e., beta-VLDL or LDL.

Genetic models of atherosclerosis, namely, the homozygote Watanabe Heritable Hyperlipidemic rabbit (WHHL) which lacks functional LDL receptors, have also been compared to cholesterol-fed rabbit models [27,28]. Like the casein-fed rabbits, plasma cholesterol was primarily distributed in LDL. In WHHL rabbits, leukocyte margination, subendothelial accumulation of isolated lipid-filled macrophages, accumulation of SMC and formation of fatty streaks occurred over the first 4 weeks of life [27]. A similar sequence of lesion formation was noted in New Zealand White rabbits fed a 0.1% to 0.2% cholesterol diet. Expansion of the lipid-filled monocyte-macrophage rich lesions, i.e., Type I-III fatty streaks, occurred during the first 6 months in both types of rabbits while complex Type V fibrous plaque lesions were noted in the WHHL and cholesterol-fed rabbits by 13 months of age [28-30]. An enrichment of cholesteryl ester, primarily cholesteryl oleate, was noted in the aorta of both animals over the course of 13 months and such a finding was consistent with the morphologic data noted above [31]. Despite the different lipoprotein distribution, one must conclude that the development of atherosclerosis in WHHL and cholesterol-fed rabbit is very similar and occurs within a similar time frame. One might propose that the WHHL rabbits may be useful to assess agents which lower plasma cholesterol by altering lipoprotein production since these animals lack functional LDL receptors. Cholesterol-fed models are less expensive and time consuming and may be manipulated by altering the level of cholesterol intake to assess the significance of graded degrees of hyercholesterolemia on cellular processes associated with lesion formation, e.g., monocyte adherence, margination and foam cell formation.

Thus far in the discussion of rabbits as models of atherosclerosis it is apparent that human-like atherosclerotic lesions can be induced by elevating plasma cholesterol levels through continuous or intermittent feeding of a cholesterol diet, a casein-enriched diet or by deleting functional LDL receptors as in the WHHL rabbit. It is also quite obvious that in such models a great deal of time is required to induce atherosclerotic lesions comparable to man, i.e., 6 months to 5 years.

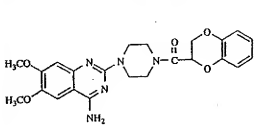
Hypercholesterolemia and mechanical denudation of the endothelium in various vascular regions of the rabbit have been utilized to develop shorter-term models of atherosclerosis with a high degree of predictability as to the location and type of atherosclerotic lesion. Acute mechanical injury of the arterial vessel wall can be achieved using a variety of methods. A balloon embolectomy catheter [32] can denude the vessel and distend the media while gentle denudation can be achieved by drawing a nylon filament over the surface of the vessel [33, 34]. Moderate injury and denudation occur following cutting of the internal elastic lamina with a metal or diamond tipped catheter [35]. Chronic endothelial damage has been shown to promote thrombosis at the catheter tip and formation of fibrous lesions [36, 37]. We have used a combination of chronic endothelial injury using a surgically implanted sterile nylon monofilament and dietary cholesterol supplementation [38, 39]. Combination of hypercholesterolemia and endothelial injury has allowed one to develop a model of atherosclerosis that is reproducible, has a high incidence of lesion formation and a predictable lesion site and type. The character of the atherosclerotic lesion is dependent upon the degree, length and type of hypercholesterolemia induced.

In summary, hypercholesterolemic rabbits are valuable models and the most widely used model for the evaluation of pharmacologic agents. Five types of human-like atherosclerotic lesions can be induced in the rabbit; however, the model is limited in that evidence of the complicated ruptured fibrous plaques cannot be found. Rabbits are also valuable for atherosclerosis research because unlike other models, atherosclerotic lesions progress even after removal of dietary cholesterol supplementation [23,40]. Evaluation of the direct antiatherosclerotic properties of hypocholesterolemic agents requires normalization of plasma cholesterol levels by diet prior to drug administration. Since rabbit atherosclerotic lesions will become more complex following cholesterol removal, agents which act by directly altering cellular processes such as ACAT inhibitors that limit macrophage accumulation can be discerned and their effect on lesion progression/regression can be monitored.

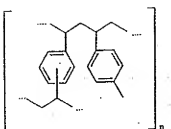
Hamster

Another model of atherosclerosis that has received recent attention is the hypercholesterolemic hamster. Male hamsters fed a 3% cholesterol, 15% butterfat diet for up to a month had elevated plasma cholesterol levels and the presence of Type I fatty dots and fatty streaks within the aortic arch [41]. Within 3 to 4 months of the very high cholesterol/fat diet, expansion of the fatty streaks into the thoracic aorta around sites of intercostal ostia was noted [41]. By 10 months of cholesterol supplementation when plasma cholesterol levels were 17-times normal, advance Type V lesions were observed in the aortic arch of the hamster but their extent was quite limited, i.e., 30% of the cross-sectional vessel surface. Feeding hamsters a 0.2% cholesterol, 10% coconut oil for 10 weeks [42] or 0.05% cholesterol, 10% coconut oil for 8 weeks [43] resulted in the accumulation of monocyte-macrophages within the aorta arch. Thus, short-term feeding of a cholesterol and either coconut oil or butterfat diet to hamsters is a model of subendothelial monocyte-macrophage foam cell formation. Atherosclerotic lesions can be found predictably within the inner curvature of the aortic arch and can be identified by staining with the lipid dye, Oil Red O. Such a model is useful due to its size for the acute evaluation of agents that may interfere with the early stages of lesion formation, e.g., monocyte adherence, transmigration and foam cell formation.

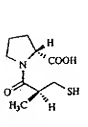
The hypercholesterolemic hamster has been used for the evaluation of numerous pharmacologic agents with varying mechanisms of action. Doxazosin Fig. (5) (23), an alpha-1 adrenergic inhibitor, and cholestyramine Fig. (5) (24) decreased the extent of Oil Red O-positive macrophage foam cells; however, one could not discern that this was a direct effect on the arterial wall because plasma cholesterol levels were



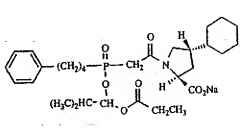
23. Doxazosin



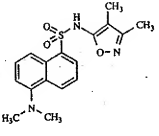
24. Cholestyramine



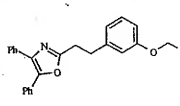
25. Captopril



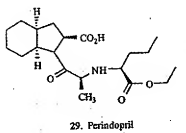
26. Fosinopril



27. BMS-182874



28. BMY-42393



29. Perindopril

Fig. (5). Broad group of compounds reported to possess antiatherosclerotic activities.

reduced by both groups and doxazosin (23) lowered blood pressure [43]. Higher doses of doxazosin (23) did not have any greater lipid lowering effect but a more marked reduction in macrophage foam cell area was noted and such data is suggestive that the compound may have a direct effect on monocyte-macrophage accumulation [42]. The HMG-CoA reductase inhibitor, lovastatin Fig. (2) (15), also was shown to reduce macrophage accumulation but again the changes were associated with a decrease in plasma cholesterol levels [44]. Inhibitors of angiotensin converting enzyme such as captopril Fig. (5) (25) without lowering plasma cholesterol and fosinopril Fig. (5) (26) by lowering LDL cholesterol, reduced aortic arch macrophage accumulation [45]. An additional study with captopril (25) and fosinopril (26) aimed at assessing the ability of these compounds to regress hamster atherosclerotic lesions was performed [46]. Both compounds were reported to reduced macrophage accumulation and thereby induce regression; however, while a group of animals was necropsied at 4 weeks to establish the degree of atherosclerosis prior to intervention only the drug treated animals were followed for an additional 6 weeks. A control designed to assess the effect of plasma cholesterol lowering or continued cholesterol feeding without intervention was not included. The ACAT inhibitor, octimibate [47] Fig. (1) (1), and endothelial subtype A receptor antagonist, BMS-182874 [48] Fig. (5) (27), both of which lowered plasma cholesterol, and the prostacyclin agonist, BMY42393 [47] Fig. (5) (28) in the absence of cholesterol lowering, have been shown to limit macrophage accumulation in the hypercholesterolemic hamster. Thus, the hypercholesterolemic hamster has proven to be a useful model for the assessment of compounds; however, the changes in plasma cholesterol and blood pressure confound the interpretation of the antiatherosclerotic data and limit one's ability to ascribe the activity to a direct effect of the compounds.

Swine

Swine are a non-rodent model of atherosclerosis in which atherosclerotic lesions have been found to develop spontaneously [49]. The pathogenesis of lesion development in pigs has been shown to closely parallel the stages of lesion formation as seen in man [50-52]. In addition, atherosclerotic lesion development can be exacerbated by combination of hypercholesterolemia and endothelial injury [53-56]. A strain of pigs with mutant apolipoprotein B alleles has been identified and these animals have been shown to be hypercholesterolemic due to defective lipoprotein clearance and prone to premature development of atherosclerosis [57-60]. Unlike the rabbit and hamster where lesions predominate in the aorta, atherosclerotic lesions have been observed in cerebral [61] and coronary [62] vessels of the pig. Thus, swine are a useful model for the evaluation of atherosclerosis from the perspective that lesions develop spontaneously, their circulatory system and localization of lesions are similar to man and the lesions are

responsive to dietary intervention by exhibiting regression after prolonged periods [63,64].

Despite their similarity to man with respect to atherosclerosis development, swine are not widely used for the evaluation of antiatherosclerotic agents. We have reported that the ACAT inhibitor, CI-976 Fig. (1) (2), blunted the progression of diet- and injury-induced atherosclerotic lesions in Yucatan miniature pigs potentially by inhibiting arterial ACAT and by lowering plasma VLDL-cholesterol levels [65]. The ACE inhibitor, perindopril Fig. (5) (29), was evaluated in hypercholesterolemic miniature pigs and noted to limit the development and monocyte-macrophage enrichment of aortic lesion; however, mean arterial blood pressure (MABP) was reduced in the animals [66]. Such reductions in MABP like the decrease in VLDL-cholesterol levels confound the interpretation of the data and limit one's ability to ascertain whether the compounds had a direct effect on lesion development. The limited a priori knowledge of a compound's effect on plasma cholesterol or blood pressure and the animal's inherent size are major disadvantages of using pigs for assessment of a compound's direct antiatherosclerotic potential. Miniature swine weigh approximately 12 kg at 4 months of age and in our studies feeding pigs a 2% cholesterol, 16% fat diet resulted in a doubling of their body weight within 4 months of diet initiation. Therefore, although swine are excellent models of atherosclerosis that mimic the human disease from the perspective of lesion pathology, such a model may be limited to evaluation of the antiatherosclerotic potential of compounds during their drug development stages rather than discovery phases.

Monkeys

Non-human primates have often been portrayed as ideal models of human atherosclerosis due to their close phylogenetic association to man. The morphologic characterization of atherosclerotic lesion progression and regression has been performed in cynomlgous (*Macaca fascicularis*)[67-69], rhesus (*Macaca mulatta*) [70], cebus (*Cebus albifrons*) [71], squirrel (*Saimiri sciureus*) [71] and pigtail (*Macaca nemestrina*) [72-75] monkeys. The pathology of atherosclerotic lesion development in various monkey species has been shown to be quite similar to man. Spontaneous development of atherosclerotic lesions is rare in non-human primates; however, like in the animals noted above cholesterol feeding has been shown to promote the development of atherosclerosis in the monkey. Experimentally induced advanced atherosclerosis in monkeys requires approximately 3 years and addition of dietary cholesterol supplementation to produce plasma cholesterol levels of between 350 and 500 mg/dl. The localization of atherosclerotic lesions was similar to man in that lesions were present in the coronary arteries, abdominal aorta and iliac arteries. Plasma cholesterol levels of 200-400 mg/dl have been noted to produce advanced Type V fibrous plaques in *Macaca nemestrina* after 3.5 years of diet [72,73]. A retrospective evaluation of cebus and squirrel monkeys administered a normal diet without cholesterol supplementation and ranging in age from 12 to 20 years highlighted the difference in susceptibility to atherosclerosis and lesion character [71]. The older squirrel monkeys were found to have advanced Type IV-V atherosclerotic lesions containing lipid enrichment and a necrotic lipid core in the abdominal aorta while lesions from ... r cebus monkeys were characterized as diffuse intimal thickening without lipid accumulation [71]. Thus, it is apparent from evaluation of monkey models of atherosclerosis that lesions of comparable character to man, swine and in some cases rabbits can be achieved; however, a combination of a lower plasma cholesterol level and a longer period of time is required for lesion progression.

Few studies have been performed in monkeys using either dietary or pharmacologic intervention to promote lesion regression. After a 30 month lesion induction phase, switching cynomologous monkeys to a chow diet initially, i.e., 6 months, results in a reduction in monocyte-macrophages and cholesteryl esters; however, intimal necrosis and free cholesterol monohydrate remain [67]. Within 12 months, the atherosclerotic lesions tended to resolve to an intimal scar with a lipid composition similar to normal vessels except for the presence of cholesterol crystals [67]. The bile acid sequestrant, cholestyramine Fig. (5) (24), the antioxidant, probucol Fig. (3) (20), either alone or upon coadministration was shown to promote atherosclerosis lesion regression in the rhesus monkeys [76] presumably due to lowering plasma cholesterol levels. Therefore, although atherosclerotic lesion development appears to mimic human disease progression, the utility of using these animals for drug discovery is limited by their availability and potential variability due to their underlying differences in age and degree of atherosclerosis progression. In addition, given the time frames required for lesion development in the monkey, rabbits fed a low cholesterol diet may be a viable substitute as shown by Wilson and colleagues [22].

Transgenic Mice

Due to advances in molecular biology and the realization that mice, in general, are normally resistant to the development of atherosclerosis [77], genetically engineered mice have been developed which are predisposed to hypercholesterolemia-induced disease. Two well-characterized transgenic mouse models of atherosclerosis are the apolipoprotein E (apoE)-deficient mouse [78-80] and the low density lipoprotein (LDL) receptor-negative mouse [81,82]. ApoE is a major component of plasma lipoproteins that has a high affinity for LDL receptors and chylomicron remnant receptors [83,84] and may be important in facilitating reverse-cholesterol transport from peripheral tissues. The apoE-deficient mice have been shown to be hypercholesterolemic, i.e., 400 to 700 mg/dl at 5 to 55 weeks of age, while maintained on a chow diet [79]. Atherosclerotic lesions develop naturally over the time frame of 11 to 64 weeks within the aortic sinus and exhibit a similar histologic appearance as Type I to V lesions. Monocyte-macrophage foam cells predominate either as individual cells or clusters in the early stages of lesion development, i.e., less than 28 weeks, while fibrosis, intimal necrosis, acellular, necrotic lipid-rich cores with evidence of cholesterol clefts can be found after 32 weeks of age [79]. A similar histologic pattern can be seen in apoE-deficient mice fed a Western-type diet, i.e., 0.15% cholesterol; however, the timecourse of lesion development is shorter and the extent of atherosclerosis is greater [80]. Cholesterol-fed apoE-deficient mice have plasma cholesterol levels of 1000 to 4400 mg/dl over 6 to 40 weeks of age. Evidence of Type IV-V complex fibrous plaques can be seen as

early as 15 weeks and the lesions are not only present in the aortic sinus but also associated with the bifurcations of such major branch vessels as the common carotids, celiac, mesenteric, renal and iliac arteries [80].

The LDL receptor-negative transgenic mouse has also been developed [81,82]. Unlike the apoE-deficient mice, atherosclerotic lesions do not occur naturally during the timeframes currently studied, i.e., 6 months [82]. Dietary supplementation with 0.15% cholesterol results in plasma cholesterol levels of 900 to 1000 mg/dl over 6 months and the development of atherosclerotic lesions within the aortic sinus. The morphologic appearance and extent of atherosclerosis in the LDL receptor-negative mouse is similar to comparably fed apoE-deficient mice; however, plasma cholesterol levels are half that noted for the apoE-deficient mice and there is greater variability in the latter mouse model [82]. Thus, both the apoE and LDL receptor-deficient mice are viable small animal models for the evaluation of atherosclerotic lesion progression.

The utility of apoE and LDL receptor-deficient mice for the evaluation of antiatherosclerotic agents has yet to be determined. Few studies have been reported which utilize these mice in drug intervention studies. The antioxidant, N,N'-diphenyl 1,4-phenylenediamine (DPPD) Fig. (3) (21) has been evaluated in apoE-deficient mice fed along with a 0.15% cholesterol diet for 6 months and was found to reduce the extent of aortic atherosclerosis by 36%, i.e., control - 22%; DPPD (21) - 14% lesion coverage [85]. In contrast, probucol Fig. (3) (20), another antioxidant with hypolipidemic properties, accelerated the development of atherosclerosis in apoE-deficient mice irrespective of whether the compound was administered in a chow or cholesterol containing diet and despite lowering plasma cholesterol level [86]. Such paradoxical observations raise an important issue relating to interpretation of the results of drug intervention studies in genetically derived mouse models. One must question the appropriateness of the model for testing the specific compound of interest. For instance, unlike the LDL receptor-negative mouse that is a model of a naturally occurring abnormality, i.e., familial hypercholesterolemia, apoE-deficient mice possess a specific genetic deletion of an apolipoprotein that may be necessary for reverse-cholesterol transport. One can argue that if a compound's antiatherosclerotic activity is mediated through apoE metabolism such a mouse model would be inappropriate for assessing the compound's activity.

Numerous other transgenic mouse models have been developed. A few transgenic mouse models of potential relevance to atherosclerosis from the perspective of lipoprotein metabolism are the human apolipoprotein B [87], apolipoprotein (a) [88], Lp(a) [89,90] and cholesteryl ester transfer protein (CETP) [91] transgenic models. In addition, one might predict that site specific deletions or overexpression of pro-atherosclerotic molecules such as adhesion molecules, growth factors, cytokines or integrins, for example, would be useful models for the assessment of direct acting antiatherosclerotic agents. A caveat to such an approach is exemplified by the comparison of the apoE- and LDL receptor-deficient mice. Both genetic defects resulted in a similar atherosclerotic lesion pathology and required some degree of hypercholesterolemia. Therefore, temporal evaluations of lesion development in the presence and absence of pharmacologic agents may be more informative in assessing whether the specific gene product/defect exacerbates disease progression and whether pathologic redundancies limit the efficacy of the specific pharmacologic entity.

Pharmacologic Intervention Studies

ACAT

Acyl-CoA:cholesterol O-acyltransferase (ACAT) is the primary enzyme responsible for the esterification of cholesterol in all mammalian cells, but under conditions of excessive cholesterol accumulation in the vascular wall, ACAT may be responsible for the generation of the hallmark of atherosclerosis, namely, the monocyte-macrophage foam cell. Since ACAT and cholesterol esterification may be considered a pro-atherogenic event, we and others have hypothesized that inhibition of arterial wall ACAT may prevent the formation of the macrophage-enriched fatty streak and the development of the clinically significant fibrous plaque. In addition, given the observations that monocyte-macrophages are localized to the potentially friable shoulder regions of atherosclerotic lesions and in association with matrix degrading enzymes, one might speculate that by limiting the accumulation of monocyte-macrophages through inhibition of ACAT one would promote the development of a stable plaque morphology.

Several inhibitors of ACAT have been evaluated in animals and they have been found to be antiatherosclerotic by measuring changes in lesion extent and/or cholesteryl ester enrichment. Schaffer and coworkers [92] reported that administration of CL277082 Fig. (1) (3) to rabbits for 16 weeks after a 10-week lesion induction phase resulted in a 49% reduction in aortic cholesteryl ester content. Cyclandalate Fig. (1) (4), a relatively weak inhibitor (IC_{50} = 80 (M) with an unknown mechanism of inhibition [93] was shown to blunt the increase in aortic total cholesterol content when given to rabbits in a low-cholesterol chow diet after a lesion induction phase [94]. A more specific and potent inhibitor of ACAT, namely, RP-70676 Fig. (1) (5), administered in a similar manner as cyclandalate (4), was reported to decrease aortic free and esterified cholesterol content by 27 to 42% [95]. In addition, melinamide [96,97] Fig. (1) (6) and the furobenzochrome Fig. (1) (7) reported by Gammill et al. [98] were shown to prevent lesion formation in cholesterol-fed rabbits. Kimura and colleagues have also reported that a series of phenylureas Fig (1) (8, 9, 10) limited the progression of aortic atherosclerotic lesions in the rabbit [99,100]. Other potent and systemically bioavailable inhibitors of ACAT, namely, E5324 [101] Fig. (1)(11) and FR145237 [102] Fig. (1)(12), significantly inhibited the progression and cholesterol enrichment of aortic atherosclerosis in rabbits. CI-976 Fig. (1)(2), a potent, systemically bioavailable inhibitor of ACAT was evaluated in hypercholesterolemic Yucatan micropigs and was noted to prevent the formation of atherosclerotic lesions [65]. Despite achieving plasma CI-976 (2) levels of 9 to 52 times the IC_{50} for inhibition of ACAT in mouse peritoneal macrophages, an accepted in vitro model of foam cell formation, one was left to conclude in this model that the antiatherosclerotic activity of the compound may be related to reductions in plasma VLDL-cholesterol since the antiatherosclerotic activity was highly correlated with plasma VLDL-cholesterol levels. Therefore, given the fact that in each of the studies cited above plasma total cholesterol levels were reduced in the animal models to the same extent or greater than

animals switched to a chow, low cholesterol diet, classification of these compounds as direct acting antiatherosclerotic agents is difficult.

Direct inhibition of arterial wall ACAT with a potent, systemically bioavailable agent although much harder to discern both preclinically and clinically may provide a greater vascular benefit than can be achieved by plasma cholesterol lowering alone. The ACAT inhibitor, CI-976 Fig. (**1**)(2), a fatty acid amide, was evaluated in a cholesterol-fed rabbit model of atherosclerosis at a dose that was bioavailable but did not lower plasma total cholesterol [39]. CI-976 (2) prevented the accumulation of monocyte-macrophages within a preestablished fibrofoamy lesion, attenuated the development of thoracic aortic fatty streak-like lesions and decreased the cholesteryl ester enrichment of the developed lesions. We have also reported that two isoxazoles Fig. (**1**)(**13, 14**) which were bioavailable based on a bioassay designed to assess plasma ACAT inhibitory bioequivalents limited the development of thoracic aortic foamy lesions but were inactive in the more fibroproliferative femoral lesions of the rabbit [103]. Others have also reported that in Watanabe heritable hyperlipidemic (WHHL) rabbits, a model of familial hypercholesterolemia lacking the LDL receptor, E5324 [104] Fig. (**1**)(**11**) and FR145237 [102] Fig. (**1**)(**12**) can limit the development of atherosclerotic lesions in the thoracic and coronary arteries in the absence of plasma cholesterol lowering. Kogushi et al. [104] have also shown that E5324 (**11**) markedly reduced aortic ACAT activity; however, such a decrease may be related to the reduction in lesion and macrophage extent and not a representation of direct arterial wall ACAT inhibition. Considering the data with CI-976 (**2**), E5324 (**11**) and FR145237 (**12**), one can conclude that ACAT inhibition has the potential to limit atherosclerosis progression by specifically affecting vascular monocyte-macrophage accumulation. However, it is also quite apparent from the various studies cited above that the experimental protocols can be quite varied.

The animal experimental protocols can be classified into several major categories. Firstly, compounds were administered either at the initiation of dietary cholesterol supplementation and the animals were necropsied after 2 to 4 months of treatment. These studies are often termed progression studies in that the effect of the compound on monocyte-macrophage accumulation or generation of Type I to Type III lesions is being studied. However, the hypocholesterolemic activity of the compounds limits the assessment of direct antiatherosclerotic activity. Secondly, compounds were given after a degree of hypercholesterolemia and atherosclerotic lesion development has been established. In most cases, animals were fed a cholesterol diet for 8 to 10 weeks and administered the various compounds either in the same cholesterol diet or a chow diet for an additional 6 to 8 weeks. Such studies can assess whether compounds can limit the further progression of a preestablished lesion and/or promote lesion regression. The ability to study lesion regression is often lost because few studies randomize animals based on their plasma total cholesterol and necropsy a group prior to drug intervention in order to assess the type, extent and composition of the lesions. Under these regression paradigms the antiatherosclerotic activity of the compound is best assessed when compared to either a group of animals switched to a chow diet or administered a cholesterol absorption inhibitor such as cholestyramine Fig. (**5**)(**24**) in order to match the degree of cholesterol lowering. Despite the extended period of hypercholesterolemia such rabbit models are still representative of early fibrofoamy lesions (Type I-III) and not a reflection of the more advanced Type IV-V fibrous plaques. Thirdly, WHHL rabbits that appear refractory to plasma cholesterol lowering caused by ACAT inhibition can be used to assess a compound's antiatherosclerotic activity. The WHHL may be a very good model for the assessment of ACAT inhibitors in that plasma total and lipoprotein cholesterol levels remain relatively constant following treatment and more advanced atherosclerotic fibrous plaque lesions may develop in the long-term. Fourthly, as reported for CI-976 (2), a combination of chronic endothelial injury and cholesterol feeding in either a progression or regression paradigm can allow one to not only assess the development and regression of atherosclerotic lesions but also determine whether the compounds have an effect on the cellular composition of a defined, well-characterized lesion with a greater than 99% incidence of occurrence. Finally, models of more advanced atherosclerosis can also be developed and used to assess whether ACAT inhibitors can limit the formation or promote the more rapid development of advanced fibrous plaques. Rabbits exposed to chronic endothelial injury within one week of study initiation and sequentially fed a cholesterol, fat diet for 9 weeks, a fat only diet for 6 weeks and various compounds for 8 additional weeks has allowed us to develop advanced fibrous plaques in the rabbit within 23 weeks and to further address the benefit of ACAT inhibitors. Therefore, numerous models have been developed specifically for testing the direct antiatherosclerotic activity of ACAT inhibitors; however, our conclusions ascribing the activity of the compound to direct inhibition of arterial ACAT is still based on circumstantial evidence.

Numerous in vitro, biochemical and pharmacokinetic studies have been performed in order to relate plasma drug levels with the compound's IC_{50} for macrophage ACAT inhibition. The basis for claiming that an ACAT inhibitor is directly antiatherosclerotic appears to be rooted in the concept that if plasma drug levels are maintained above the IC_{50} for inhibition of macrophage ACAT and plasma total and lipoprotein cholesterol levels are unchanged then the compound has direct antiatherosclerotic properties. One assumes that the compound at steady state will partition into the various atherosclerotic lesions and inhibit macrophage ACAT. Direct measurement of arterial wall ACAT and vessel drug levels have been performed [104]. Given the observations that ACAT inhibitors limit arterial wall macrophage enrichment, i.e., a source of arterial ACAT, one must be cautious in interpreting a reduction in arterial wall ACAT activity as evidence for direct ACAT inhibition. A more plausible explanation is that there is a decrease in the amount of ACAT enzyme due to a reduction in macrophage accumulation. Standardization of atherosclerotic lesion size and cellular composition prior to acute administration of the ACAT inhibitor and assessment of ACAT activity may provide more definitive proof of direct arterial wall ACAT inhibition. However, an absence of ACAT inhibition may be misleading in that during the microsome isolation procedures compounds may be diluted out of the sample. Quantification of vessel drug levels is not only problematic and their accuracy can be questioned for the same reasons as noted above for the measurement of ACAT activity but also drug extraction efficiency and metabolism become an issue. Although still circumstantial another marker of arterial ACAT inhibition is the cholesteryl ester content of the vessel wall under comparable

levels of plasma total cholesterol exposure and atherosclerotic lesion/macrophage extents.

HMG-CoA Reductase Inhibitors

3-hydroxy-3-methylglutaryl coenzyme A reductase (HMG-CoA reductase), in addition to being the rate limiting enzyme in the cholesterol biosynthetic pathway, is involved in the regulation of receptors for LDL-cholesterol [105]. In experimental animals [106], and patients with heterozygous familial hypercholesterolemia [107] inhibition of hepatic HMG-CoA reductase leads to an increased number of LDL receptors on the cell surface, which ultimately results in an enhanced clearance of plasma LDL and a reduction in plasma total cholesterol levels. However, in nonfamilial hypercholesterolemic and familial combined hyperlipidemic patients, HMG-CoA reductase inhibitors lower plasma cholesterol by inhibiting lipoprotein production [108]. Reductions in plasma total cholesterol of over 30% and in LDL-cholesterol of 40% have been observed in clinical trials with various doses of atorvastatin [109] Fig. (2)(16), lovastatin [110] Fig. (2)(15), pravastatin [111] Fig. (2)(17), and simvastatin [112] Fig. (2)(18). In addition, the recent Scandinavian Simvastatin Survival Study (4S) [113] has shown that lowering plasma cholesterol by 35% with diet and simvastatin (18) significantly reduces the risk of mortality by 30%, coronary heart disease mortality by 42%, and incidence of nonfatal myocardial infarction by 37%. The West of Scotland Study (WOSCOPS) has demonstrated that lowering plasma LDL-cholesterol by 26% with diet and pravastatin (17) significantly reduced the risk of mortality from definite coronary events by 31% [114]. Thus, the data in man indicate that inhibitors of HMG-CoA reductase by reducing plasma cholesterol may limit the development of atherosclerosis and reduce the risk of mortality.

Several animal studies have also shown that lovastatin (15) and pravastatin (17) can attenuate atherosclerotic lesion development when plasma total and LDL-cholesterol are reduced [115-118], and that atorvastatin (16) can limit lesion development independent of changes in plasma cholesterol [119]. Due to the potent hypolipidemic activity of HMG-CoA reductase inhibitors, the assessment of these compound's direct antiatherosclerotic potential in preclinical models of atherosclerosis is difficult. However, comparison of compounds with a similar plasma total cholesterol exposure may allow one to assess whether an agent has any inherent antiatherosclerotic properties. For instance, we reported that in a cholesterol-fed rabbit model of lesion progression, lovastatin (15), pravastatin (17) and atorvastatin (16) reduced plasma total cholesterol exposure over the course of the experiment by 37% to 43% [119]. Given the linear relationship between plasma cholesterol and atherosclerosis extent noted previously [120], one might expect that the degree and composition of the atherosclerotic lesions would be similar amongst the three treatment groups. Despite equal plasma cholesterol levels, pravastatin (17) and lovastatin (15) had no effect on thoracic aortic lesion extent or iliac-femoral cross-sectional lesion area. In contrast, atorvastatin (16) reduced the thoracic aortic lesion extent from 44% to 19% and iliac-femoral lesion area by 67%. Thus, we concluded that atorvastatin can directly limit atherosclerosis lesion progression.

Evaluation of the va... as HMG-CoA reductase inhibitors in a rabbit model of atherosclerosis lesion progression highlights the power of the experimental design in formulating an interpretation of the data. Given the fact that plasma cholesterol levels were reduced, an analysis of a subset of compounds with the same cholesterol exposure allowed one to assess their relative antiatherosclerotic activity and also ascribe the activity to a direct effect on the lesion. Establishment of the effect of lipid-lowering on atherosclerosis development is an important factor when assessing compound efficacy. Addition of control treatments such as cholestyramine Fig. (5)(24), a non-absorbable resin, or diets containing graded cholesterol contents are methods for assessing the antiatherosclerotic activity of a compound at defined plasma cholesterol levels. Reductions in lesion size, extent or composition above that predicted for a given plasma cholesterol level may indicate that the compound is directly altering a pro-atherogenic event.

Comparison of biochemical, morphologic and morphometric results may allow one to establish the consistency of the effect and to identify potential mechanisms for the observed antiatherosclerotic activity. For instance[119], not only did atorvastatin Fig. (2)(16) decrease the extent of thoracic aortic atherosclerosis but also reduced the cholesteryl ester content of the thoracic aorta, a secondary marker that is reflective of the lesion extent and composition, i.e., lipid , monocyte-macrophage enrichment. Examination of the histopathology of the atherosclerotic lesions and morphometric changes following treatment allowed one to discern potential mechanisms responsible for the observed antiatherosclerotic activity. For example, pravastatin Fig. (2)(17) had no effect on lesion or monocyte-macrophage area while atorvastatin Fig. (2)(16) reduced both parameters. One might conclude from these data that pravastatin (17) lacked sufficient plasma drug levels or did not penetrate the arterial wall and that atorvastatin (16) by directly limiting lesion size through inhibition of smooth muscle cell migration and proliferation indirectly reduced macrophage accumulation. The later hypothesis is consistent with observations made by others which indicate that HMG-CoA reductase inhibitors in tissue culture limit SMC proliferation [121-123] and migration [124] by interfering with isoprenoid synthesis [125].

Therefore, by controlling for the degree of plasma cholesterol lowering and combining multiple efficacy parameters, one might not only be able to discriminate the direct antiatherosclerotic activity of a compound from that due to plasma cholesterol lowering but also by evaluating the structure of the atherosclerotic lesions identify potential mechanisms which can be tested in vitro or in appropriate animal models.

Anti-Oxidants and 15-Lipoxygenase Inhibitors

Steinberg and colleagues [126] have reported that oxidatively modified LDL may be important in the progression of atherosclerosis due to the observations that oxidized LDL is cytotoxic, chemotactic and chemostatic. Oxidative modification of insudant plasma lipoproteins is presumably an extracellular event [126] and the resulting oxidized lipoproteins have been implicated in the regulation of chemokines ,[127] and pro-atherogenic adhesion molecules [128]. Both apolipoprotein B [129-132], the major protein in LDL, and lipid peroxides have been localized to atherosclerotic lesions [133]. Oxidatively

modified LDL or such oxidation products as malondialdehyde-conjugated LDL or 4-hydroxynonenal-conjugated LDL have been localized to WHHL rabbits [134-136]. Thus, one can conclude that oxidation of lipoproteins may be important in the development of atherosclerosis and that general antioxidants may be antiatherosclerotic in both man and models of atherosclerosis.

Several studies investigating the antiatherosclerotic activity of general antioxidants have been performed in New Zealand white rabbits [137-140], WHHL rabbits [141,142], pigs [143] and monkeys [76] under a variety of experimental conditions. In cholesterol-fed rabbits, butylated hydroxytoluene (BHT) Fig. (3)(22), vitamins E plus C, vitamins E plus A and probucol Fig. (3)(20) limited the development of thoracic aortic lesions [137-140, 144-146]. Probucol (20) has been shown by numerous individuals to reduce the extent, cholesterol enrichment and cross-sectional lesion size of atherosclerotic lesions in WHHL rabbits [141,142], balloon-injured normocholesterolemic pigs [143] and hypercholesterolemic monkeys [76]. Close examination of the lesion histopathology revealed that probucol (20) reduced the extent of atherosclerosis by decreasing the abundance of monocyte-macrophages within the lesion [146]. Mechanistic studies in rabbits fed cholesterol for a short time period, i.e., 5 wks, indicated that probucol (20) can limit the adhesion of monocytes to the endothelial cell surface. The single study in coronary artery balloon-injured pigs also indicated that probucol (20) can limit the development of primarily fibroproliferative lesions through presumably affecting SMC migration and proliferation [143]. Thus, one can conclude that antioxidants and specifically, probucol (20), can limit the development and cellular composition of atherosclerotic lesions in various animal models of atherosclerosis irrespective of whether the compound was administered to animals with or without pre-established lesions.

In most of the studies noted above, plasma cholesterol lowering was minimal so attempts to identify surrogate markers of vascular efficacy of the various antioxidants were made. Resistance of lipoproteins to oxidation was a major surrogate marker used by most investigators [141,142]. Measurements of vascular reactivity were also made [147,148]. In hypercholesterolemic rabbits, probucol (20) treatment preserved endothelial function and vascular rings upon exposure to acetylcholine in organ culture were shown to relax normally [147]. The improved vascular responsiveness is quite remarkable and one can conclude that antioxidants may improve vascular function; however, while in both studies plasma total cholesterol levels were relatively constant among the control and probucol-treated (20) groups, one study [148] reported that the cholesterol content of vessels from the drug-treated group was reduced. Since atherosclerosis is comprised of multiple stages and drugs such as probucol (20) can alter the type as well as cellular and lipid composition of the atherosclerotic lesions, correlation of pathology with functional changes is important in the assessment of drug efficacy. Experimental protocols can be designed to assess the inherent activity of compounds to promote vasorelaxation. Given the observation that some agents lower plasma or vascular cholesterol levels, administration of agents to normocholesterolemic animals or atherosclerotic animals where plasma cholesterol levels are normalized by diet may allow one to assess whether the compound has a direct effect on vascular relaxation either in the presence or absence of underlying disease.

Based on the ... hology data and the localization of epitopes of oxidize LDL within the arterial wall, one can suggest that general antioxidants may be useful antiatherosclerotic agents. However, specific inhibitors of the oxidation process may allow one to target a specific pro-atherogenic process and to better characterize the compound's activity in models of atherosclerosis. A new enzyme specific target, namely arachidonate 15-lipoxygenase (15-LO), has emerged [149]. Arachidonate 15-lipoxygenase is a lipid-peroxidizing enzyme that is also present in atherosclerotic lesions. Investigators have found the 15-LO gene [150], stereospecific products of the 15-LO enzyme [151] and coincident localization of 15-LO mRNA, protein and epitopes of oxidized LDL within macrophage-rich areas of atherosclerotic lesions [149]. We have identified a specific inhibitor of 15-LO, namely, PD146176 Fig. (3)(19), and have evaluated the compound in several models of atherosclerosis [152,153].

Evaluation of PD146176 (19) in the hypercholesterolemic rabbit under three specific experimental paradigms has allowed us to conclude that in the absence of lowering plasma total and lipoprotein cholesterol levels PD146176 (19) can attenuate the development of diet induced atherosclerotic lesions through specific inhibition of monocyte-macrophage accumulation. In addition, PD146176 (19) can limit the development and macrophage enrichment of pre-established atherosclerotic lesions. PD146176 (19) administered to rabbits coincident with a cholesterol diet reduced the gross extent of foamy lesions (Type I-III lesions) within and cholesterol enrichment of the thoracic and abdominal aorta [152]. PD146176 (19) administered to rabbits coincident with a cholesterol diet and induction of a chronic endothelial injury not only reduced the progression of foamy thoracic lesions but also specifically limited the accumulation of monocyte-macrophages within a fibrofoamy iliac-femoral lesion without affecting the overall lesion size [153]. PD146176 (19) administered after establishment of fibrofoamy Type IV lesions through a combination of chronic endothelial injury and dietary cholesterol supplementation reduced the extent, cross-sectional area and monocyte-macrophage content of the more advanced Type V fibrous plaque [153]. In all three studies, assessment of plasma total and lipoprotein levels, vascular lipid content and histologic evidence for the presence of 15-LO in the lesions were necessary to corroborate the findings and maintain the implication that 15-LO was involved. Thus, these data highlight the antiatherosclerotic potential of a specific 15-LO inhibitor.

The brief summary of the antiatherosclerotic effects of PD146176 (19) can also be used to exemplify the power of the animal models of atherosclerosis. The simplest model, a rabbit fed a 0.25% cholesterol, 3% peanut, 3% coconut oil diet illustrated that the compound can prevent the formation of cholesteryl ester enriched Type III foamy atherosclerotic lesions. One might propose that evaluation of rabbits fed cholesterol for shorter periods of time would allow one to assess whether the observed antiatherosclerotic activity was due to reduced monocyte adherence. In the second rabbit model, induction of a fibrofoamy lesion by chronic endothelial injury allowed one to build upon the first observation and suggest that the compound specifically limited monocyte-macrophage accumulation because the absolute lesion cross-sectional area was unchanged. In the most complex model, one was able to assess whether PD146176 (19) could limit the progression of the disease to a fibrous plaque or promote regression of a

preestablished fibrofoamy lesion. In addition, one can obtain such mechanistic information as to the involvement of 15-LO in advanced atherosclerosis and whether further monocyte-macrophage enrichment can be blunted.

Conclusions

Atherosclerotic lesion development can be divided into six histologically distinct stages and five dynamic phases. Specifically, atherosclerotic lesion progression in man involves episodes of SMC proliferation, extracellular matrix deposition and remodeling, lipid infiltration, endothelial cell-monocyte interactions, monocyte migration into the intima, monocyte-macrophage foam cell formation, necrotic lipid-rich core formation, calcium deposition, neovascularization, mural microthrombi and occlusive acute thrombosis. Given the complexity of atherosclerotic lesion development in man, the challenge exists to develop animal models that closely mimic the human disease. One must accept, however, that there is no one perfect animal model that completely replicates the stages of human atherosclerosis but that the models are useful in studying specific pathologic processes associated with the disease. Hypercholesterolemic rabbits either with or without endothelial injury are valuable models and the most widely used model for the evaluation of pharmacologic agents. Five types of human-like atherosclerotic lesions can be induced in the rabbit; however, the model is limited in that evidence of the plaque rupture cannot be found. Hypercholesterolemic hamsters are a model of an early pro-atherogenic event, namely, subendothelial monocyte-macrophage foam cell formation. Swine are a useful model for the evaluation of atherosclerosis from the perspective that lesions develop spontaneously, their circulatory system and localization of lesions are similar to man and the lesions are responsive to dietary intervention by exhibiting regression after prolonged periods. Non-human primates have often been portrayed as ideal models of human atherosclerosis due to their close phylogenetic association to man; however, lesions of comparable character to man can be induced more efficiently and over shorter time periods in swine and in some cases rabbits through a combination of hypercholesterolemia and endothelial injury. Numerous transgenic mouse models have been developed in recent years. A common finding among the various mouse models of atherosclerosis is that a similar atherosclerotic lesion pathology develops and all require some degree of hypercholesterolemia. Therefore, temporal evaluations of lesion development in the presence and absence of pharmacologic agents may be more informative in assessing whether the specific gene product/defect exacerbates disease progression and whether pathologic redundancies limit the efficacy of the specific pharmacologic entity. Based on evaluation of the various animal models and pharmacologic agents, one can conclude that: (1) each animal model provides insight into specific aspects of the disease process; (2) a hypercholesterolemic state is required in all models for the development of atherosclerosis; (3) discrimination of the *direct* antiatherosclerotic activity of a compound from it's *indirect* activity requires one to limit the number of confounding factors, e.g., hypocholesterolemic and antihypertensive effect; (4) combination of biochemical, morphologic and morphometric measures allows one to both validate the antiatherosclerotic effect and define potential mechanisms; (5) reducing monocyte-macrophage involvement irrespective of mechanism or animal model effectively limits the development of atherosclerotic lesions.

Abbreviations

ACAT	=	Acyl-coenzyme A:cholesterol O-acyltransferase
HMG-CoA reductase	=	3-Hydroxy-3-methylglutaryl coenzyme A
15-LO	=	15-Lipoxygenase
SMC	=	Smooth muscle cells
VCAM-1	=	Vascular cell adhesion molecule-1
VLDL	=	Very low density lipoproteins
LDL	=	Low density lipoproteins
WHHL	=	Watanabe heritable hyperlipidemic rabbit
ACE	=	Angiotensin converting enzyme
MABP	=	Mean arterial blood pressure
ApoE	=	Apolipoprotein E
CETP	=	Cholesteryl ester transfer protein
4S	=	Scandinavian Simvastatin Survival Study
WOSCOP	=	The West of Scotland Study

Acknowledgement

I would like to thank Dr. D. Robert Sliskovic for his kind invitation to write this article and for his help in preparation of the figures depicting chemical structures of the compounds cited in the manuscript.

References

[1] Tracy, R.E. *Lab. Invest.*, 1979, 41, 546.

[2] Constantinides, P. J. *Ultrastruct. Res.*, 1966, 6, 1.

[3] Ridolfi, R.L.; Hutchins, G.M. *Am. Heart J.*, 1977, 93, 468.

[4] Cornhill, J.F.; Herderick, E.E.; Stary, H.C. *Monogr. Atheroscler.*, 1990, 15, 13.

[5] Cornhill, J.F.; Barrett, W.A.; Herderick, E.E.; Mabley, R.W.; Fry, D.L. *Arterioscler.*, 1985, 5, 415.

[6] Stary, H.C.; Chandler, A.B.; Glagov, S.; Guyton, J.R.; Insull, W. Jr.; Rosenfeld, M.E.; Schaffer, S.A.; Schwartz, C.J.; Wagner, W.D.; Wissler, R.W. *Circ.*, 1994, 89, 2462.

[7] Fuster, V. *Circ.*, 1994, 90, 2126.

[8] Smith, E.B.; Evans, P.H.; Downham, M.D. *J. Atheroscler. Res.*, 1967, 7, 171.

[9] Kume, N.; Cybulsky, M.I.; Gimbrone, M.A. *J. Clin. Invest.*, 1992, 90, 1138.

[10] Cybulsky, M.I.; Gimbrone, M.A. *Science*, 1991, 252, 788.

[11] Krause, B.R.; Sliskovic, D.R.; Bocan, T.M.A. *Exp. Opin. Invest. Drugs*, 1995, 4, 353.

[12] Anitschkow, N. Beitr. Pathol. *Anat. Allgem. Pathol.*, 1913, 56, 379.

[13] Jokinen, M.P.; Clarkson, T.B.; Prichard, R.W. *Exp. Mol. Pathol.*, 1985, 42, 1.

[14] Prior, J.T.; Kurtz, D.M.; Ziegler, D.D. *Arch. Pathol.*, 1961, 71, 672.

[15] Kroon, P.A.; Thompson; Chao, Y. *Atherosclerosis*, 1985, 56, 323.

[16] Prior, J.T.; Kurtz, D.M.; Ziegler, D.D. *Arch. Pathol.*, 1961, 71,82.

[17] Kritchevsky, D.; Tepper, S.A.; Vesselinovitch, D.; Wissler, R.W. *Atherosclerosis*, 1971, 14, 53.

[18] Kritchevsky, D.; Tepper, S.A.; Kim, H.K.; Story, J.A.; Vesselinovitch, D.; Wissler, R.W. *Exp. Molec. Pathol.*, 1976, 24, 375.

[19] Ho, K.J.; Pang, L.C.; Taylor, C.B. *Atherosclerosis*. 1974, 19, 561.

[20] Ross, A.C.; Minick, C.R.; Zilversmit, D.B. *Atherosclerosis*, 1978, 29, 301.

[21] Mahley, R.W.; Innerarity, T.L.; Rall, S.C. Jr; Weisgraber, K.H. *Ann. NY Acad. Sci.*, 1985, 454, 209.

[22] Wilson, R.B.; Miller, R.A.; Middleton, C.C.; Kinden, D. *Arteriosler.*, 1982, 2, 228.

[23] Constantinides, P.; Booth, J.; Carlson, G. *Arch. Pathol.*, 1960, 70, 712.

[24] Constantinides, P. *J. Atherosclerosis Res.*, 1961, 1, 374.

[25] Daley, S.J.; Herderick, E.E.; Cornhill, F.; Rogers, K.A. *Arteriosler. Thromb.*, 1994, 14, 95.

[26] Daley, S.J.; Klemp, K.F.; Guyton, J.R.; Rogers, K.A. *Arterioscler. Thromb.*, 1994, 14, 105.

[27] Rosenfeld, M.E.; Tsukada, T.; Gown, A.M.; Ross, R. *Arterioscler.*, 1987, 7, 9.

[28] Rosenfeld, M.E.; Tsukada, T.; Chait, A.; Bierman, E.L.; Gown, A.M.; Ross, R. *Arterioscler.*, 1987, 7, 24.

[29] Tsukada, T.; Rosenfeld, M.; Ross, R.; Gown, A.M. *Arterioscler.*, 1986, 6, 601.

[30] Shiomi, M.; Ito, T.; Tsukada, T.; Yata, T.; Ueda, M. *Arterioscler. Thromb.*, 1994, 14, 931.

[31] Rosenfeld, M.E.; Chait, A.; Bierman, E.L.; King, W.; Goodwin, P.; Walden, C.E.; Ross, R. *Arterioscler.*, 1988, 8, 338.

[32] Stadius, M.L.; Rowan, R.; Fleishhauer, J.F.; Kernoff, R.; Billingham, M.; Gown, A.M. *Arterioscler. Thromb.*, 1992, 12, 1267.

[33] Reidy, M.A.; Harker, L.A.; Schwartz, S. *Fed. Proc.*, 1983, 42, 771.

[34] Reidy, M.A.; Yoshida, K.; Harker, L.A.; Schwartz, S.M. *Arteriosler.*, 1986, 6, 305.

[35] Bjorkerud, S.; Bondjers, G. *Atherosclerosis*, 1971, 13, 355.

[36] Moore, S. *Lab. Invest.*, 1973, 29, 478.

[37] Moore, S.; Hanahan, R.J.; Singal, D.P.; Gaudie, J.; Blajchman, M.A.; Roberts, R.S. *Thrombos. Haemostasis (Stuttgart)*, 1976, 35, 70.

[38] Bocan, T.M.A.; Bak Mueller, S.; Uhlendorf, P.D.; Ferguson, E.; Newton, R.S. *Expt. Molec. Pathol.*, 1991, 54, 201.

[39] Bocan, T.M.A.; Bak Mueller, S.; Uhlendorf, P.D.; Newton, R.S.; Krause, B.R. *Arterioscler. Thromb.*, 1991, 11, 1830.

[40] Friedman, M.; Byers, S.O. *Am. J. Pathol.*, 1963, 43, 349.

[41] Nistor, A.; Bulla, A.; Filip, D.A.; Radu, A. *Atherosclerosis*, 1987, 68, 159.

[42] Foxall, T.L.; Shwaery, G.T.; Stucchi, A.F.; Nicolosi, R.J.; Wong, S.S. *Am. J. Pathol.*, 1992, 140, 1357.

[43] Kowala, M.C.; Nunnari, J.J.; Durham, S.K.; Nicolosi, R.J. *Atherosclerosis*, 1991, 91, 35.

[44] Otto, J.; Ordovas, J.M.; Smith, D.; van Dongen, D.; Nicolosi, R.J.; Schaefer, E.J. *Atherosclerosis*, 1995, 114, 19.

[45] Kowala, M.C.; Grove, R.I.; Aberg, G. *Atherosclerosis*, 1994, 108, 61.

[46] Kowala, M.C.; Recce, R.; Beyer, S.; Aberg, G. *J. Cardiovascular Pharm.*, 1995, 25, 179.

[47] Kowala, M.C.; Mazzucco, C.E.; Hartl, K.S.; Seiler, S.M.; Warr, G.A.; Abid, S.; Grove, R.I. *Arterioscler. Thromb.*, 1993, 13, 435.

[48] Kowala, M.C.; Rose, P.M., Stein, P.D.; Goller, N.; Recce, R.; Beyer, S.; Valentine, M.; Barton, D.; Durham, S.K. *Am. J. Pathol.*, 1995, 146, 819.

[49] Gottlieb, H; Lalich, J.J. *Am. J. Pathol.*, 1954, 30, 851.

[50] Moreland, A.F.; Clarkson, T.B.; Lofland, H.B. *Arch. Pathol.*, 1963, 76, 99.

[51] French, J.E.; Jennings, M.A.; Florey, H.W. *Ann. N Y Acad. Sci.*, 1965, 127, 780.

[52] Reitman, J.S.; Mahley, R.W.; Fry, D.L. *Atherosclerosis*, 1982, 43, 119.

[53] Nam, S.C.; Lee, W.M.; Jarmolych, J.; Lee, K.T.; Thomas, W.A. *Exp. Molec. Pathol.*, 1973, 18, 369.

[54] Lee, W.M.; Lee K.T. *Exp. Molec. Pathol.*, 1975, 23, 491.

[55] Fritz, K.E.; Daoud, A.S.; Augustyn, J.M.; Jarmolych, J. *Exp. Molec. Pathol.*, 1980, 32, 61.

[56] Dov Gal, D.V.M.; Rongione, A.J.; Slovenkai, G.A.; DeJesus, S.T.; Lucas, A.; Fields, C.D.; Isner, J.M. *Am. Heart J.*, 1990, 119, 291.

[57] Rapcz, J.; Hasler-Rapacz, J.; Taylor, K.M.; Checovich, W.J.; Attie, A.D. *Science*, 1986, 234, 1573.

[58] Lowe, S.W.; Checovich, W.J.; Rapacz, J.; Attie, A.D. *J. Biol. Chem.*, 1988, 263, 15467.

[59] Checovich, W.J.; Fitch, W.L.; Krauss, R.M.; Smith, M.P., Rapacz, J.; Smith, C.L.; Attie, A.D. *Biochem.*, 1988, 27, 1934.

[60] Prescott, M.F.; McBride, C.H.; Hasler-Rapacz, L.; Von-Linden, J.; Rapacz, J. *Am. J. Pathol.*, 1991, 139.

[61] Detweiler, D.K.; Ratcliffe, H.L.; Luginbuhl, H. *Ann. N. Y. Acad. Sci.*, 1968, 149, 868.

[62] Reddick, R.L.; Read, M.S.; Brinkhous, K.M., Bellinger, D.; Nichols, T.; Griggs, T.R. *Arterioscler.*, 1990, 10, 541.

[63] Daoud, A.S.; Jarmolych, J.; Augustyn J.M.; Fritz, K.E.; Singh, J.K.; Lee, K.T. *Arch. Pathol. Lab. Med.*, 1976, 100, 372.

[64] Fritz, K.E.; Augustyn, J.M.; Jarmolych, J.; Daoud, A.S.; Lee, K.T. *Arch. Pathol. Lab. Med.*, 1976, 100, 380.

[65] Bocan, T.M.A.; Bak Mueller, S.; Uhlendorf, P.D.; QuenbyBrown, E.; Mazur, M.J.; Black, A.E. *Atherosclerosis*, 1993, 99, 175.

[66] Charpiot, P.; Rolland, P.H.; Friggi, A.; Piquet, P.; Scalbert, E.; Bodard, H.; Barlatier, A.; Latrille, V.; Tranier, P.; Mercier, C.; Luccioni, R.; Calaf, R.; Garcon, D. *Arterioscler. Thromb.*, 1993, 13, 1125.

[67] Small, D.M.; Bond, M.G.; Waugh, D.; Prack, M.; Sawyer, J.K. *J. Clin. Invest.*, 1984, 73, 1590.

[68] Stary, H.C.; Malinow, M.R. *Atherosclerosis*, 1982, 43, 151.

[69] Kramsch, D.M.; Hollander, W. *Expt. Molec. Pathol.*, 1968, 9, 1.

[70] DePalma, R.G.; Klein, L.; Bellon, E.M.; Koletsky, S. *Arch. Surg.*, 1980, 115, 1268.

[71] Hoover, G.A.; Nicolosi, R.J.; Camp, R.R.; Hayes, K.C. *Arterioscler.*, 1982, 2, 252.

[72] Masuda, J.; Ross, R. *Arterioscler.*, 1990, 10, 164.

[73] Masuda, J.; Ross, R. *Arterioscler.*, 1990, 10, 178.

[74] Faggiotto, A.; Ross, R.; Harker, L. *Arterioscler.*, 1984, 4, 323.

[75] Faggiotto, A.; Ross, R. *Arterioscler.*, 1984, 4, 341.

[76] Wissler, R.W.; Vesselinovitch, D. *Appl. Pathol.*, 1983, 1, 89.

[77] Paigen, B.; Ishida, B.Y.; Verstuyft, J.; Winters, R.B.; Albee, D. *Arterioscler.*, 1990, 10, 316.

[78] Plump, A.S.; Smith, J.D.; Hayek, T.; Aalto-Setala, K.; Walsh, A.; Verstuyft, J.G.; Rubin, E.M.; Breslow, J.L. *Cell*, 1992, 71, 343.

[79] Reddick, R.L.; Zhang, S.H.; Maeda, N. *Arterioscler. Thromb.*, 1994, 14, 141.

[80] Nakashima, Y.; Plump, A.S.; Raines, E.W.; Breslow, J.L.; Ross, R. *Arterioscler. Thromb.*, 1994, 14, 133.

[81] Ishibashi, S.; Goldstein, J.L.; Brown, M.S.; Herz, J.; Burns, D.K. *J. Clin. Invest.*, 1994, 93, 1885.

[82] Tangirala, R.K.; Rubin, E.M.; Palinski, W. *J. Lipid Res.*, 1995, 36, 2320.

[83] Mahley, R.W. *Science*, 1988, 240, 622.

[84] Brown, M.S.; Goldstein, J.L. *Science*, 1986, 232, 4.

[85] Tangirala, R.K.; Casanada, F.; Miller, E.; Witzum, J.L.; Steinberg, D.; Palinski, W. *Arterioscler. Thromb. Vasc. Biol.*, 1995, 15, 1625.

[86] Zhang, S.H.; Reddick, R.L.; Avdievich, E.; Surles, L.K.; Jones, R.G.; Reynolds, J.B.; Quarfordt, S.H.; Maeda, N. *J. Clin. Invest.*, 1997, 99, 2858.

[87] Purcell-Huynh, D.A.; Farese, R.V., Jr.; Johnson, D.F.; Flynn, L.M.; Pierotti, V.; Newland, D.L.; Linton, M.F.; Sanan, D.A.; Young, S.G. *J. Clin. Invest.*, 1995, 95, 2246.

[88] Lawn, R.M.; Wade, D.P.; Hammer, R.E.; Chiesa, G.; Verstuyft, J.G.; Rubin, E.M. *Nature*, 1992, 360, 670.

[89] Mancini, F.P.; Newland, D.L.; Mooser, V.; Murata, J.; Marcovina, S.; Young, S.G.; Hammer, R.E.; Sanan, D.A.; Hobbs, H.H. *Arterioscler. Thromb. Vasc. Biol.*, 1995, 15, 1911.

[90] Callow, M.J.; Verstuyft, J.; Tangirala, R.; Palinski, W.; Rubin, E.M. *J. Clin. Invest.*, 1995, 96, 1639.

[91] Maroti, K.R.; Castle, C.K.; Boyle, T.P.; Lin, A.H.; Murray, R.W.; Melchior, G.W. *Nature*, 1993, 364, 73.

[92] Schaffer, S.A.; Bloom, J.D.; DeVries, V.G.; Dutia, M.; Karocs, A.S. Jr.; Largis, E. In *Atherosclerosis VII*; Fidge, N.H.; Nestel, P.J., Eds; Elsevier Science Publishers B.V.: Amsterdam, 1986, pp 633-636.

[93] Heffron, F.; Middleton, B.; White, D.A. *Biochem. Pharm.*, 1990, 39, 575.

[94] Middleton, B.; Middleton, A.; White, D.A.; Bell, G.D. *Atherosclerosis*, 1984, 171, 171.

[95] Ashton, M.J.; Bridge, A.W.; Bush, R.C.; Dron, D.I.; Harris, N.V.; Jones, G.D.; Lythgoe, D.J.; Riddell, D.; Smith, C. *Bioorg. Med. Chem. Lett.* 1992, 2, 375.

[96] Fukushima, H.; Aono, S.; Nakamura, Y.; Endo, M.; Imai, T. *J. Atheroscler. Res.*, 1969, 10, 403.

[97] Fukushima, H.; Toki, K.; Nakatani, H. *J. Atheroscler. Res.*, 1969, 9, 57.

[98] Gammill, R.B.; Bell, F.P.; Bell, L.T.; Bisaha, S.N.; Wilson, G.J. *J. Med. Chem.* 1990, 33, 2686.

[99] Kimura, T.; Takase, Y.; Hayashi, K.; Tanaka, H.; Ohtsuka, I.; Saeki, T.; Kogushi, M.; Yamada, T.; Fujimori, T.; Saitou, I.; Akasaka, K. *J. Med. Chem.*, 1993, 36, 1630.

[100] Kimura, T.; Watanabe, N.; Matsui, M.; Hayashi, K.; Tanaka, H.; Ohtsuka, I.; Saeki, T.; Kogushi, M.; Kabayashi, H.; Akasaka, K.; Yamagishi, Y.; Saitou, I.; Yamatsu, I. *J. Med. Chem.*, 1993, 36, 1641.

[101] Tanaka, H.; Ohtsuka, I.; Kogushi, M.; Kimura, T.; Fujimor, T.; Saeki, T.; Hayashi, K.; Kobayashi, H.; Yamada, T.; Hiyoshi, H.; Saito, I. *Atherosclerosis*, 1994, 107, 187.

[102] Matsuo, M.; Ito, F.; Konto, A.; Aketa, M.; Tomoi, M.; Shimomura, K. *Biochim. Biophys. Acta*, 1995, 1259, 254.

[103] White, A.D.; Purchase II, C.F.; Picard, J.A.; Anderson, M.K., Bak Mueller, S.; Bocan, T.M.A.; Bousley, R.F.; Hamelehle, K.L.; Krause, B.R.; Lee, P.; Stanfield, R.L.; Reindel, J.F. *J. Med. Chem.*, 1996, 39, 3908.

[104] Kogushi, M.; Tanaka, H.; Ohtsuka, I.; Yamada, T.; Kobayashi, H.; Saeki, T.; Takada, M.; Hiyoshi, H.; Yanagimachi, M.; Kimura, T.; Yoshitake, S.; Saito, I. *Atherosclerosis*, 1996, 124, 203.

[105] Brown, M.S.; Goldstein, J.L. *Science*, 1986, 232, 34.

[106] Kovanen, P.T.; Bilheimer, D.W.; Goldstein, J.L.; Jaramillo, J.J.; Brown, M.S. *Proc. Natl. Acad. Sci. USA*, 1981, 78, 1194.

[107] Bilheimer, D.W.; Grundy, S.M.; Brown, M.S.; Goldstein, J.L. *Proc. Natl. Acad. Sci. USA*, 1983, 80, 4124.

[108] Arad, Y.; Ramakrishnan, R.; Ginsberg, H.N. *Metabolism*, 1992, 41, 487.

[109] Nawrocki, J.W.; Weiss, S.R.; Davidson, M.H.; Sprecher, D.L.; Schwartz, S.S.; Jupien, P.J.; Jones, P.H.; Haber, H.E.; Black, D.M. *Arterioscler. Thromb. Vasc. Biol.*, 1995, 15, 678.

[110] Walker, J.F. *Drugs*, 1988, 36, 83.

[111] McTavish, D.; Sorkin, E.M. *Drugs*, 1991, 42, 65.

[112] Todd, P.A.; Goa, K.L. *Drugs*, 1990, 40, 583.

[113] Lancet, 1994, 344, 1383.

[114] Shepherd, J.; Cobbe, S.M.; Ford, I.; Isles, C.G.; Lorimer, A.R.; MacFarlane, P.W.; McKillop, J.H.; Packard, C.J. *N. Engl. J. Med.*, 1995, 333, 1301.

[115] Kritchevsky, D.; Tepper, S.A.; Klurfeld, D.M. *Pharm. Res. Comm.*, 1981, 13, 921.

[116] Kobayashi, M.; Ishida, F.; Takahashi, T.; Taguchi, K.; Watanabe, K.; Ohmura, I.; Kamei, T. *Jpn. J. Pharmacol.* 1989, 49, 125.

[117] Zhu, B.Q.; Sievers, R.E.; Sun, Y.P.; Isenberg, W.M.; Parmley, W.W. *J. Cardiovas. Pharm.*, 1992, 19, 246.

[118] Shiomi, M.; Ito, T.; Watanabe, Y.; Tsujita, Y.; Kuroda, M.; Arai, M.; Fukami, M.; Fukushige, J.; Tamura, A. *Atherosclerosis* 1990, 83, 69.

[119] Bocan, T.M.A.; Mazur, M.J.; BakMueller, S.; QuenbyBrown, E.; Sliskovic, D.R.; O'Brien, P.M.; Creswell, M.W.; Lee, H.; Uhlendorf, P.D.; Roth, B.D.; Newton, R.S. *Atherosclerosis*, 1994, 111, 127.

[120] Bocan, T.M.A.; BakMueller, S.; Mazur, M.J.; Uhlendorf, P.D.; QuenbyBrown, E.; Kieft, K.A. *Atherosclerosis*, 1993, 102, 9.

[121] Corsini, A.; Mazzotti, M.; Raiteri, M.; Soma, M.; Fumagalli, R.; Paoletti, R. *XI Intl. Symp. Drugs Affecting Lipid Metabolism*, 1992, 7.

[122] Corsini, A.; Raiteri, M.; Soma, M.; Fumagalli, R.; Paoletti, R. *Pharmacol. Res.*, 1991, 23, 173.

[123] Falke, P.; Mattiasson, I.; Stavenow, L.; Hood, B. *Pharm. Toxicol.*, 1989, 64, 173.

[124] Hidaka, Y.; Tomoyo, E.; Yonemoto, M.; Kamei, T. *Atherosclerosis*, 1992, 95, 87.

[125] Corsini, A.; Bernini, F.; Quarato, P.; Donetti, E.; Bellosta, S.; Fumagalli, R.; Paoletti, R.; Soma, V.M. *Cardiology*, 1996, 87, 458.

[126] Steinberg, D.; Parthasarathy, S.; Carew, T.E.; Khoo, J.C.; Witzum, J.L. *New Engl. J. Med.*, 1989, 320, 915.

[127] Rajavashisth, T.B.; Andalibi, A.A.; Territo, M.C.; Berliner, J.A.; Navab, M.; Fogelman, A.M.; Lusis, A.J. *Nature*, 1990, 344, 254.

[128] Kume, N.; Cybulsky, M.I.; Gimbrone, M.A. *J. Clin. Invest.*, 1992, 90, 1138.

[129] Kao, C.Y.; Wissler, R.W. *Exp. Molec. Pathol.*, 1965, 4, 465.

[130] Knieriem, H.J.; Kao, C.Y.; Wissler, R.W. *Arch. Pathol. Lab. Med.*, 1967, 84, 118.

[131] Yomantas, S.; Elner, V.M.; Schaffner, T.; Wissler, R.W. *Arch. Pathol. Lab. Med.*, 1984, 108, 374.

[132] Bocan, T.M.A.; Brown, S.A.; Guyton, J.R. *Arterioscler.*, 1988, 8, 499.

[133] Glavind, J.; Hartmann, S.; Clemmesen, I.; Jessan, K.E.; Dam, H. *Acta Pathol. Microbiol. Scand.*, 1952, 3-, 1.

[134] Boyd, H.C.; Gown, A.M.; Wolfbauer, G.; Chait, A. *Am. J. Pathol.*, 1989, 135, 815.

[135] Haberland, M.E.; Fong, D.; Cheng, L. *Science*, 1988, 241, 215.

[136] Palinski, W.; Rosenfeld, M.E.; Yla-Herttuala, S.; Gurtner, G.C.; Socher, S.S.; Butler, S.W.; Parthasarathy, S.; Carew, T.E.; Steinberg, D.; Witzum, J.L. *Proc. Natl. Acad. Sci. USA*, 1989, 86, 1372.

[137] Daugherty, A.; Zweifel, B.S.; Schonfeld, G. *Br. J. Pharmacol.*, 1989, 98, 612.

[138] Tawara, K.; Ishihara, M.; Ogawa, H.; Tomikawa, M. *Jpn. J. Pharmacol.*, 1986, 41, 211.

[139] Stein, Y.; Stein, O.; Delplanque, B.; Fesmire, J.D.; Lee, D.M.; Alaupovic, P. *Atherosclerosis*, 1989, 75, 145.

[140] Carew, T.E.; Schwenke, D.C.; Steinberg, D. *Proc. Natl. Acad. Sci. USA*, 1987, 84, 7725.

[141] Daugherty, A.; Zweifel, B.S.; Schonfeld, G. *Br. J. Pharmacol.*, 1991, 103, 1013.

[142] Kita, T.; Nagano, Y.; Yokode, M.; Ishii, K.; Kume, N.; Ooshima, A.; Kawai, C. *Proc. Natl. Acad. Sci. USA*, 1987, 84, 5928.

[143] Schneider, J.E.; Berk, B.C.; Gravanis, M.B.; Santoian, E.C.; Cipolla, G.D.; Tarazona, N.; Lassegue, B.; King, S.B., 3rd *Circ.*, 1993, 88, 628.

[144] Bjorkhem, I.; Henriksson-Freyschuss, A.; Breuer, O.; Diczfalusy, U.; Berglund, L.; Henriksson, P. *Arterioscler. Thromb.*, 1991, 11, 15.

[145] Brattsand, R. *Atherosclerosis*, 1975, 22, 47.

[146] Bocan, T.M.A.; BakMueller, S. QuenbyBrown, E.; Uhlendorf, P.D.; Mazur, M.J.; Newton, R.S. *Expt. Molec. Pathol.*, 1992, 57, 70.

[147] Keaney, J.F., Jr.; Xu, A.; Cunningham, D.; Jackson, T.; Frei, B.; Vita, J.A. *J. Clin. Invest.*, 1995, 95, 2520.

[148] Del Rio, M.; Challa, T.; Ruiz, E.; Tejerina, T. *Br. J. Pharmacol.*, 1996, 118, 1639.

[149] Yla-herttuala, S.; Rosenfeld, M.E.; Parthasarathy, S.; Glass, C.K.; Sigal, E.; Witzum, J.L.; Steinberg, D. *Proc. Natl. Acad. Sci. USA*, 1990, 87, 6959.

[150] Yla-herttuala, S. *Herz*, 1992, 17, 270.

[151] Kuhn, H.; Belkner, J.; Zaiaa, S.; Fahrenklemper, T.; Wohlfeil, S. *J. Exp. Med.*, 1994, 179, 1903.

[152] Sendobry, S.M.; Cornicelli, J.A.; Welch, K.; Bocan, T.; Tait, B.; Trivedi, B.K.; Colbry, N.; Dyer, R.D.; Feinmark, S.J.; Daugherty, A. *Br. J. Pharmacol.*, 1997, 120, 1199.

[153] Bocan, T.M.A.; Rosebury, W.S.; BakMueller, S.; Kuchera, S.; Welch, K.; Daugherty, A.; Cornicelli, J.A. *Atherosclerosis*, 1997, in press.